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Published in:
Journal of Hazardous Materials

Link to article, DOI:
[10.1016/j.jhazmat.2016.03.067](https://doi.org/10.1016/j.jhazmat.2016.03.067)

Publication date:
2017

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Mackevica, A., Olsson, M. E., & Hansen, S. F. (2017). The release of silver nanoparticles from commercial toothbrushes. *Journal of Hazardous Materials*, 322(Part A), 270-275.
<https://doi.org/10.1016/j.jhazmat.2016.03.067>

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The release of silver nanoparticles from commercial toothbrushes

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Abstract

The use of silver nanoparticles (NPs) in commercial products has become increasingly common in the past decade, mostly due to their antimicrobial properties. Using Ag NP-containing articles may lead to particle release, which raises concern of human and environmental safety. The published literature addressing particle release is scarce, especially when it comes to quantifying exposure to NPs specifically. In this study, we have experimentally investigated the release of total Ag and Ag NP from commercially available toothbrushes i.e. biodegradable toothbrushes for adults and toothbrushes for children. Toothbrushes were immersed and abraded in tap water for 24 hours corresponding to more than the whole intended usage time of a toothbrush. The total amount of released Ag was quantified by inductively coupled plasma – mass spectrometry (ICP-MS) analysis, and the Ag NPs were characterized by single particle ICP-MS and transmission electron microscopy (TEM). The median size of the released Ag NPs ranged from 42-47 nm, and the maximum total Ag release was 10.2 ng per toothbrush. The adult toothbrushes were generally releasing more total Ag and NPs than children toothbrushes. In conclusion, our results indicate that the use of Ag NP-impregnated toothbrushes can cause consumer as well as environmental exposure to Ag NPs.

Keywords: Silver nanoparticles, Consumer products, Nanoparticle release, Consumer exposure

1. Introduction

There has been increased use of engineered nanoparticles (NPs) in consumer products in the last decade due to their unique properties and wide range of applications. Specifically silver NPs are used as an antimicrobial and antifungal agent for a wide variety of products, including food contact materials, textiles, various baby products, and toothbrushes [1, 2]. The addition of Ag NPs enables manufacturers to advertise their products as more hygienic, in some cases even claiming that the same antimicrobial Ag NP technology is used as in hospitals.

The use of such products may cause the release of silver in form of ions and/or NPs, which can subsequently lead to consumer and environmental exposure. The knowledge concerning exposure to NPs is very limited due to the fact that most research has been focusing on potential hazardous effects exerted by NPs [3, 4, 5]. The issue of exposure to NPs has been insufficiently addressed in the literature so far, and the need to get more experimental data in this regard has been illustrated by a number of researchers (*e.g.* [6, 7]). The characterization and quantification of NP release could provide data for consumer and environmental exposure estimation using various guidelines, such as those developed by European Chemicals Agency [8] or the US Environmental Protection Agency [9].

There is information available in the literature regarding Ag release from various Ag NP-containing consumer products, such as paints [10], food containers [11, 12, 13], baby products [14], textiles [15, 16, 17, 18], band-aids [19] and medical supplies (catheters) [20]. As pointed out by a recent review by Mackevica & Hansen [7], there have been only 76 publications in total reporting release from NP-containing solid articles by year 2015. 34 of these 76 studies addressed release from Ag NP-containing products. However, the nanoparticle release from most consumer articles has been poorly investigated, and when it comes to experimental assessment of release from NP-containing products the results are usually presented as total Ag release rather than NP release. Focusing solely on total Ag release may overlook the NP-specific exposure and related effects. The difficulty acquiring data on NP release can mainly be

attributed to the lack of established techniques for quantitative NP characterization, and due to the difficulty of working with complex matrices in which the NPs could be released.

The need of obtaining data for NP release during the entire life cycle of the product and multiple use scenarios has been highlighted repetitively in the literature [7, 16, 17, 18, 21]. For instance, Mackevica & Hansen [7] have highlighted the importance of investigating NP release from commercial articles over the time course of their intended use, taking into account consumer-relevant conditions that could lead to particle release and subsequently exposure.

In order to address this research need, we investigated silver NP release from commercial toothbrushes for adults and children, and analyzed the total Ag release as well as quantified the particulate Ag NP release. To the best of our knowledge, this is the first study addressing silver NP release from commercially available toothbrushes, and the first study to experimentally determine the release of Ag NPs through the entire intended usage period of the product.

2. Materials & methods

2.1 Selection of products

Two different brands of toothbrushes were selected for the release experiments: biodegradable toothbrushes for adults (further referred to as A samples) and baby toothbrushes for 3-6 year old kids (further referred to as B samples). Both toothbrushes are listed in The Nanodatabase and advertise the presence of Ag NPs in the product for antibacterial properties [1].

2.1 Chemical analysis of total silver content

As the toothbrush manufacturers were stating that the bristles were impregnated with Ag NPs, the total Ag content in the toothbrushes was analyzed by acid digestion of the bristles. The acid digestion was performed using 6mL HNO₃ and 2mL H₂SO₄, which has been previously reported to be effective to digest Ag

NP-containing plastic [22]. Samples were microwave digested (Multiwave 3000, Anton-Paar) at 210° C for 35 min. If the digestion was incomplete, the procedure was repeated one more time. Digested samples were further diluted with Milli-Q water and analyzed using ICP-MS (7700x, Agilent Technologies). Currently there is no certified standard reference material available for Ag NPs embedded in plastic, so the Ag recovery was verified by standard addition using 30 nm silver nanoparticles (Cline Scientific, Sweden).

2.2 The experimental setup for release testing

The Ag release from the toothbrushes was tested in 20mL plastic vials which were filled with 15mL of tap water (water hardness 1.0 – 1.2 mg/L CaCO₃ (18 - 21° dH), pH 7 - 8.5). The volume of water added to the vials was chosen so that it was sufficient to immerse the head of the toothbrush and the toothbrushes were fixed to rotating rods (120 rounds per minute) to simulate brushing movement. The test vials were furthermore chosen so that their diameter was small enough for the bristles of the toothbrushes to come in contact with the inside wall of the vial to simulate abrasion during brushing. A schematic representation of the experimental setup is shown in Figure 1. Sampling was done at different time points (2 min, 4 min, 6 min, 2 h, 8 h, 16 h and 24 h), and for each time point the toothbrushes were transferred to fresh vials with 15 mL tap water. Pure tap water was tested at each sampling time to ensure that it was not containing Ag.

The samples were analyzed by ICP-MS in the single particle mode (spICP-MS) immediately after sampling, without any sample preparation. spICP-MS analysis was performed applying 3 ms dwell time, 7 mm sample cone depth and having a sampling time of 90 s with sample uptake rate of 0.317 mL/min. The particle size and number calculations were done according to a previously published method [23], assuming that the particles are spherical.

The samples for total Ag ICP-MS analysis were prepared by digestion with nitric acid (3.75 mL sample, 1.25 mL 65% HNO₃) for at least 24 h at room temperature in the dark. Thereafter the digested samples were diluted with Milli-Q water. Similar as for total Ag in bristles analysis described above, 30 nm Ag particles were used as digestion control.

2.3 Electron microscopy imaging of released particles

Transmission Electron Microscopy (TEM) was used to do the imaging of the Ag NPs released from the toothbrushes. Because tap water creates CaCO_3 particles when drying, making it more difficult to find Ag particles, the samples for TEM analysis were prepared in Milli-Q water. Specifically, the toothbrushes were immersed in Milli-Q water for 24 h, and thereafter approximately 3.5 μL of the sample was transferred to a copper grid covered with carbon film (Agar Scientific) and dried. TEM (Tecnai G2, FEI) was operated at 200 keV accelerating voltage and the presence of Ag particles was confirmed by energy dispersive X-ray spectroscopy (EDS) detector (Oxford Instruments).

3. Results and discussion

The analysis of the total Ag content showed that adult toothbrushes (A) contained around 28 μg Ag per toothbrush, while the Ag content in toothbrushes for kids (B) was found to be below detection limit (Table 1). Adult toothbrushes were also containing about twice as much bristles by weight in comparison to children toothbrushes, meaning that a larger number of bristles were exposed to tap water, and higher Ag content was potentially available for release.

The spICP-MS measurements from the release experiment revealed that there was measurable Ag NP release for all the toothbrushes tested. Pure tap water was checked at each sampling event as well and showed no Ag content. The detection limit of the spICP-MS method was calculated to be 35 nm. The linear particle count range for spICP-MS measurements was measured to be from 2 - 9 $\times 10^6$ particles/L, corresponding to 3.5 -200 ng Ag/L using 60 nm Ag NPs (Sigma Aldrich). The median diameter of the detected particles released from toothbrush samples was varying between 42 - 47 nm (Table 2), assuming that particles are of spherical shape. The size distributions of cumulated particle release (Figure 2) showed a similar size distribution pattern for all six toothbrushes tested, indicating that there was a rather low abundance of released particles in sizes above 60 nm. This observation might be an indication that a similar

size of Ag NPs was used to impregnate the bristles for both brands of toothbrushes. Even though each toothbrush shows a slightly different NP release pattern, baby toothbrushes (B) generally show the highest release within the first 6 minutes of exposure (Figure 3), and showing considerably lower NP release thereafter, with having little or no Ag NP release at the 24 hour sampling point. The rapid initial release might be an indication that most particles may be loosely bound to the surface of the bristles, and there is no more Ag NPs available for release afterwards. The adult toothbrushes (A), however, show continuous Ag NP release up to 16 h of testing period, at which point the particle release is becoming less than 10^6 particles/L over the final 8 hours of testing (at 24 h sampling point). Continuous Ag NP release can be related to the relatively high initial Ag content in adult toothbrushes ($\sim 28 \mu\text{g}$ per adult toothbrush in comparison to $< 0.07 \mu\text{g}$ per children toothbrush) and the greater weight of bristles, which may provide a larger surface area for Ag NP release.

When comparing the total Ag NP release over time, it becomes apparent that adult toothbrushes are releasing substantially more particles compared to baby toothbrushes (Figure 3 A), where the release after 24 h is ranging from 9.4 to 20.3 and from 3.6 to 6.6 million particles/L, respectively (Table 2). Even though the total number of Ag NPs released is notably different for B and E samples, the quantitative particle release during the first 6 min does not show a clear difference between the two types of toothbrushes (Figure 3 B), meaning that in the first few uses the exposure would be similar from both adult (A) and baby (B) toothbrushes. The initial release from first few minutes of abrasion and exposure to tap water is most likely a result of desorption of surface-bound particles, and as the total weight of the bristles per toothbrush is nearly twice higher for adult toothbrushes (A) than baby toothbrushes (B), it might mean that B samples have a higher fraction of Ag NPs on bristle surface than E samples.

Unlike the particulate Ag release, the total Ag release is showing a very similar release pattern over time for both adult (A) and children (B) toothbrushes. The total silver release is showing a higher initial release for the first 6 min of testing for all six samples (Figure 4), similarly to Ag NP measurement, but thereafter the

total Ag release continues in a slower rate until it reaches a plateau at around 16 h of exposure to tap water for both brands of toothbrushes. One possible explanation for this might be that in the first 6 minutes the bristles release most of the bigger particles or the particles that are more loosely bound to the bristles, and later on the toothbrushes continuously (up to 16 h) release smaller Ag nanoparticles and/or ionic Ag, which could not be quantified by spICP-MS.

The trend of having a declining release of total Ag from plastic articles after multiple uses has been observed also by von Goetz *et al.* [12] and Echegoyen and Nerin [20] as well when testing Ag release from food containers. It may be an indication that Ag NPs have been weakly bound to the polymer surface and thus can be easily desorbed, which might partially explain our findings. However, in the case of toothbrushes, it is not only the contact with a liquid that facilitates the Ag release, but also abrasion facilitated by the contact with the polymer surface of the vials. Ag NPs can be released as free particles or attached to or embedded into pieces of polymer matrix, and larger polymer pieces will not be detected by spICP-MS.

Electron microscopy imaging (TEM-EDS) was used to provide a qualitative insight into the shape and size of the particles in an attempt to complement the results obtained from spICP-MS analysis. Unfortunately, due to the relatively low concentrations of Ag released, it was rather difficult to find any Ag NPs on the TEM grids. However, a few particles with Ag were found released from A toothbrush sample (Figure 5). The obtained TEM images indicate that Ag nanoparticles may occur still embedded in a matrix (Figure 5 D), or in aggregates of up to 100 nm with primary particle sizes of around 20 nm to less than 10 nm (Figure 5 A, B and C). The observed particles seem to be of spherical shape, thus verifying the spICP-MS data interpretation which assumes spherical shape of the particles to calculate the particle size. The particle chemical identity was confirmed by EDS analysis (Figure 6) and indicated presence of Ag. Unfortunately, it was not possible to find any Ag particles in the TEM sample from B toothbrushes, which means the shape and size of the released particles could not be confirmed to complement the spICP-MS data.

When comparing the total and particulate Ag release, it becomes apparent that around 1-3 % of all measured Ag is in particulate form, A toothbrushes having a slightly higher fraction of particulate Ag (2.0 – 2.8 %) in comparison to B samples (0.9 – 2.1 %) (Table 2). However, it must be noted that the fraction could be significantly higher if we could quantitatively measure smaller size particles. Another cause of imprecise estimate of the particulate Ag release may be Ag NPs attached to pieces of polymer matrix (as shown in Figure 5 D), which would prevent them from being analyzed by spICP-MS as it requires a complete ionization in the plasma.

To the best of our knowledge, this is the first scientific study addressing Ag NP release from toothbrushes, and the first study to cover the entire intended usage period of the product. The 24 hours of testing corresponds to 720 times of tooth brushing, assuming that each instance takes 2 min. According to the American Dental Association [24], it is recommended to change a toothbrush every 3-4 months. Assuming 4 months use of a toothbrush and brushing twice a day for 2 min, it results in 8 h of toothbrush use in total. The results reported in this study show that even after 8 h of use, the two brands of Ag-enabled toothbrushes tested continue to release Ag (as shown in Figure 3 and Figure 4). Measuring the Ag NP release in more complex matrices such as saliva or saliva mixed with toothpaste would provide a more realistic scenario for Ag NP exposure and based on our findings, we would recommended that such studies be undertaken. Saliva has a pH of around 7 [25], which is close to that of tap water used in this study, so it would probably not have large influence to the behavior and dissolution of Ag NPs in comparison to tap water medium. Toothpaste, however, can be more acidic and have a much more complex composition. Depending on the brand, they can have pH ranging from 4 – 7 [26], which could highly influence the dissolution of Ag NPs.

The maximum observed release per toothbrush was 10.2 ng in total (sample A1), which is most probably a negligible amount to pose risk to human or environmental health by toothbrushes alone. However, the human and environmental risk assessment of Ag NPs is full of uncertainties, because the toxicological

effects of Ag NPs are not yet fully understood [27, 28]. When it comes to assessing the efficacy of the toothbrushes for killing bacteria, the baby toothbrushes would most likely not be able to kill bacteria, as the minimum inhibitory concentrations (MIC) for Ag NPs (70 nm) to *E. Coli* start from 12.5 µg/L [29], which is magnitudes higher than the total Ag release observed from this study. Other studies have noted even higher MIC of different sizes of Ag NPs (7, 29, 89 nm) to *E. Coli* ranging from around 6 to 12 mg/L [30]. For ionic silver the MIC is lower, starting already from 0.5 µg/L for lower bacterial cell densities, which is quite close to the total Ag release observed in this study, indicating that these toothbrushes might be able to kill bacteria to some extent, especially given that these toothbrushes are meant to kill bacteria when not being used, i.e. having very little liquid on the bristles for a longer time, which could result in higher silver concentrations.

Moreover, the total Ag release for A toothbrush samples is less than 0.1 % of the measured total Ag content in A toothbrushes. The small fraction of Ag released indicates that most of the Ag is embedded in the polymer matrix and is not easily desorbed or released, meaning that most Ag stays in the toothbrushes and would most likely end up in solid waste. Further research would be necessary to estimate the fate of remaining Ag in the used toothbrushes in the end-of-life phase in their life cycle.

4. Conclusions

In conclusion, our experimental results show that Ag NPs can be released from commercially available toothbrushes and can lead to potential consumer oral exposure and environmental exposure. From the two commercially available toothbrushes that were tested, the adult toothbrushes showed slightly higher Ag release both in terms of total Ag release and Ag NP release. The general trend was that particle release substantially declined after the first 6 minutes of testing, and the total Ag release (i.e. ionic Ag and/or smaller Ag nanoparticles) reached a plateau after 16 h of testing. The median size of the particles was similar to all the toothbrushes tested and was observed to be 43 -47 nm.

For both toothbrush brands the total Ag release was in levels of ng/L, which indicates close to negligible human and environmental exposure from toothbrushes alone. However, the uncertainties regarding human and environmental risk assessment of Ag NPs implies that the question of “safe levels” of Ag NP exposure remains open.

Acknowledgements

This project has received funding from the European Union’s Seventh Framework Programme [FP7/2007-2013] under EC-GA No. 604305 ‘SUN’. We sincerely thank our lab technicians Susanne Kruse and Sinh Hy Nguyen for their contribution to this work.

Competing interests

The authors declare that there are no competing interests.

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Tables

Table 1: Total Ag content in adult (A) and children (B) toothbrushes (n=3).

	Whole toothbrush	Bristles	Ag content	
	g	g	µg/g bristles	µg/toothbrush
A	16.07 ± 0.11	0.71 ± 0.01	39.16 ± 0.91	27.89 ± 0.62
B	13.53 ± 0.04	0.34 ± 0.01	< 0.21	< 0.07

Table 2: Accumulated total and particulate Ag release from adult (A) and children (B) toothbrushes in tap water after 24h.

	Total Ag release (24h)		Particulate Ag release (24h)			Particulate Ag	Median size
	ng/toothbrush	ng/L	ng/toothbrush	ng/L	10 ⁶ part/L	%	nm
A	9.39 ± 1.11	626 ± 74	0.23 ± 0.06	15.4 ± 4.2	14.2 ± 5.5	2.4 ± 0.4	46.8 ± 0.7
B	6.64 ± 1.26	444 ± 84	0.09 ± 0.04	5.9 ± 2.1	5.2 ± 1.6	1.4 ± 0.6	45.0 ± 2.1

Figures

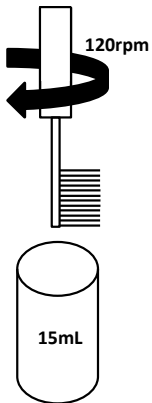


Figure 1: Schematic representation of the experimental setup.

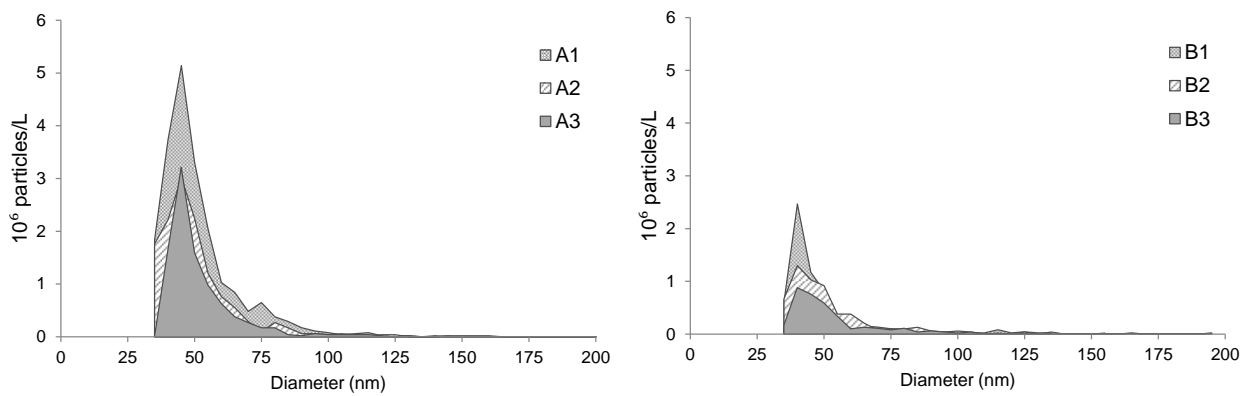


Figure 2: Cumulated released silver nanoparticle size distribution after 24 hours of testing. A: adult toothbrushes, B: baby toothbrushes. Control (pure tap water) not included.

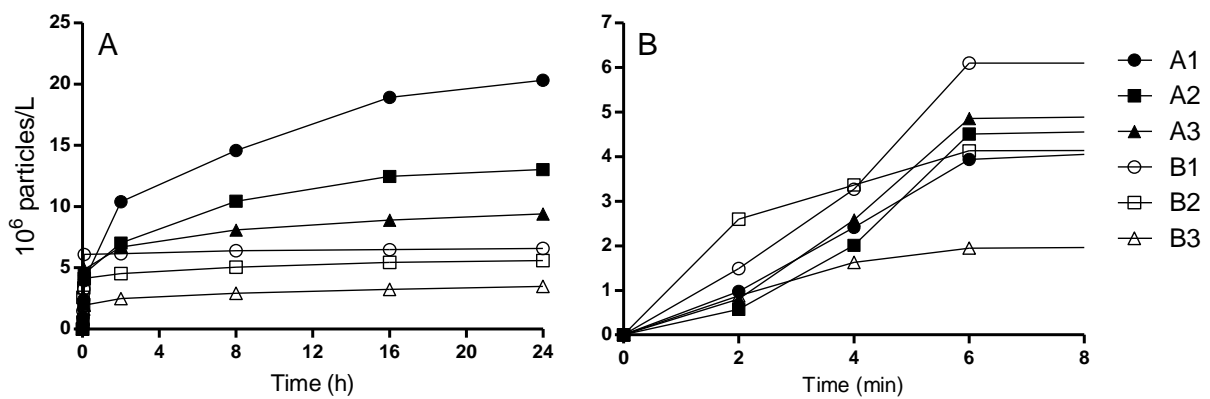


Figure 3: Particulate silver release over time measured by sp ICP-MS. A: adult toothbrushes, B: baby

toothbrushes. Control (pure tap water) not included. Graph A: Silver release over 24 hours, Graph B: Silver release for the first 8 minutes.

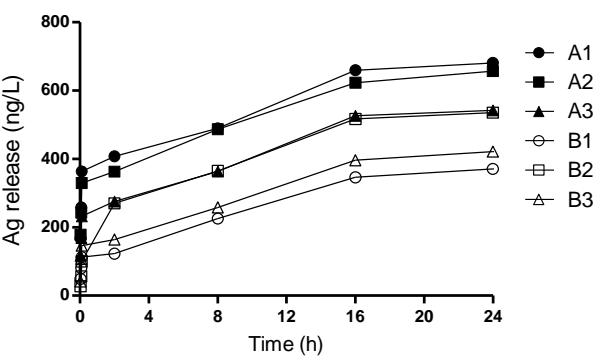


Figure 4: Total silver release over time measured by ICP-MS after acid digestion. A: adult toothbrushes, B: baby toothbrushes. Control (pure tap water) not included.

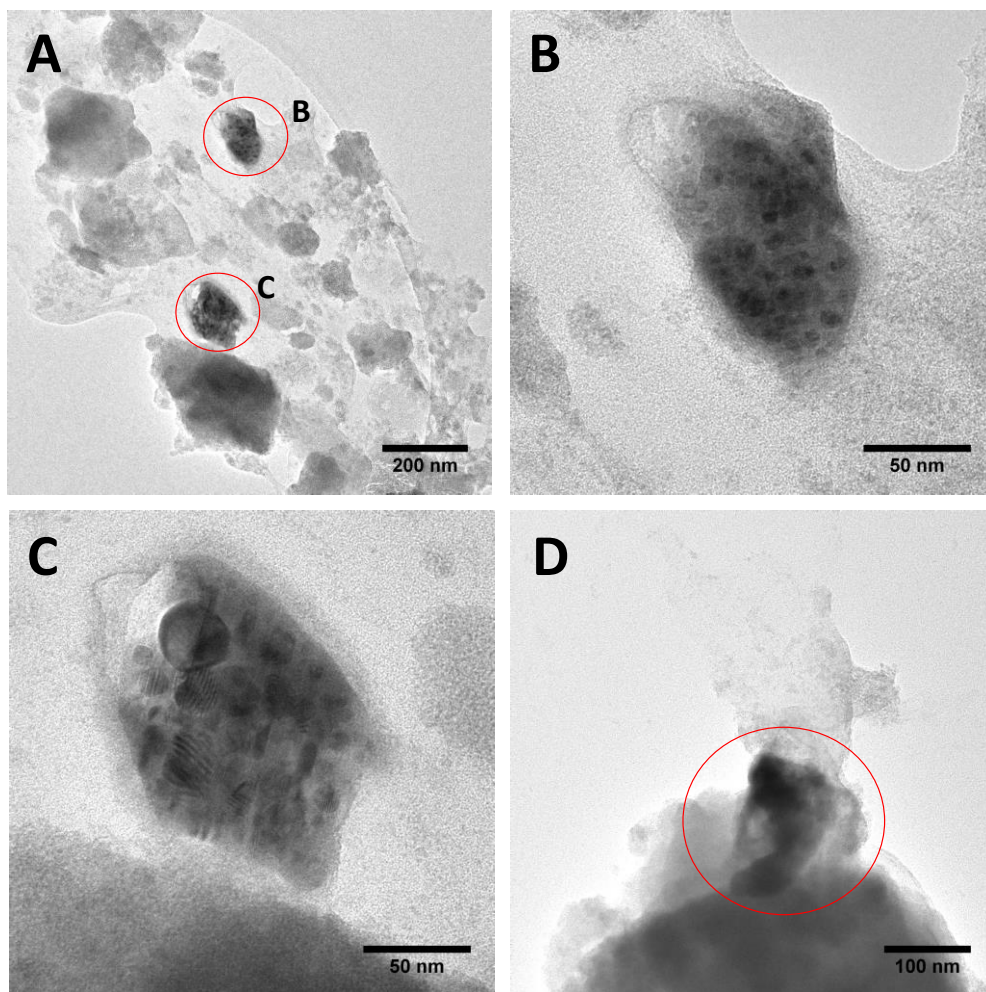


Figure 5: TEM images from particles released from adult toothbrush (A). The red circles in A and D indicate presence of silver (confirmed by EDS). The B and C images are magnifications from particles indicated in A image.

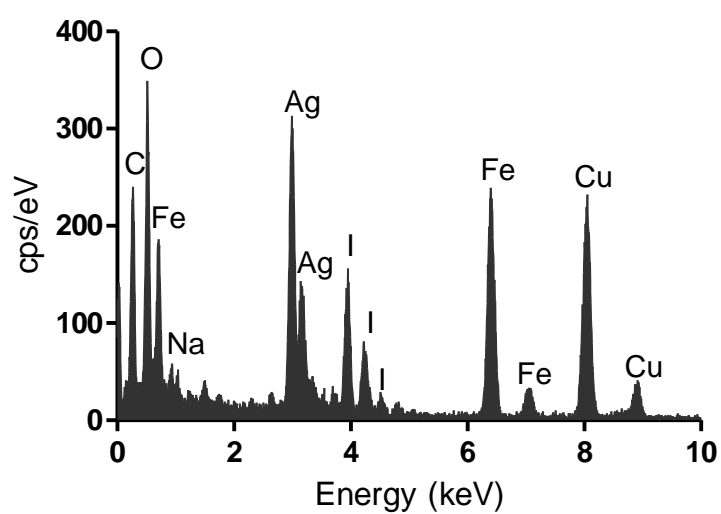


Figure 6: EDS spectrum from particle released from adult toothbrush (A), corresponding to the image shown in Figure 5 C.